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THE INFLUENCE OF SUB-TOXIC DOSE XENOBIOTIC ON IMMUNE RESPONSE OF EXPERIMENTAL ANIMALS

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Summary

The polyester is widely used in all sectors of the economy. The polyester influence on the cellular and humoral immunity has been required an integrated research. The actual medical-biological problem is immune deficiency indicators and they need to substantiate during immune toxicological research. One of the scientific directions is the investigation of polyols influence on immunobiological resistance of the human organism. The results can be used for developing of methods to increase the non-specific reactivity.

This study examines mechanisms of cellular and humoral immunity disorders of mice and rats after prolonged oral administration of Laprol- 604. Oral consumption of Laprol-604 has been shown to result in inhibition of cellular and humoral immunity of laboratory animals. The study has been performed as part of the research topic of Kharkiv Medical Academy of Postgraduate Education «Cellular-molecular mechanisms of inflammation associated with chronic diseases», the state registration number 015U001186.

Keywords: polyester, Laprol-604, humoral immunity, cellular immunity, spleen, lymph nodes.

ВПЛИВ СУБТОКСИЧНИХ ДОЗ КСЕНОБІОТИКІВ НА ІМУННУ ВІДПОВІДЬ У ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН

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Резюме

Поліестер широко використовується у всіх секторах економіки. Вплив поліестеру на вираженість клітинного та гуморального імунітет вимагає комплексного дослідження. Важливою медико-біологічною проблемою є показники імунодефіциту, що і було вивчено при імунних токсикологічних дослідженнях. Одним з наукових напрямків є вивчення впливу поліолів на імунобіологічну резистентність організму людини. Отримані результати можуть бути використані для розробки методів підвищення неспецифічної реактивності.

У даній роботі розглядаються механізми порушень клітинного і гуморального імунітету у мишей і щурів після тривалого перорального введення Лапрола-604. Показано, що пероральне введення Лапрола-604 призводить до пригнічення клітинного і гуморального імунітету у тварин. Дослідження було проведено в рамках науково-дослідної теми Харківської медичної академії післядипломної освіти «Клітинно-молекулярні механізми запалення, пов'язані з хронічними захворюваннями» (номер державної реєстрації 015U001186).

Ключові слова: поліестер, Лапрол-604, гуморальний імунітет, клітинний імунітет, селезінка, лімфатичні вузли.

ВЛИЯНИЕ СУБТОКСИЧЕСКИХ ДОЗ КСЕНОБИОТИКОВ НА ИММУННЫЙ ОТВЕТ У ЭКСПЕРИМЕНТАЛЬНЫХ ЖИВОТНЫХ

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Харьковская медицинская академия последипломного образования

Резюме

Полиэстер широко используется во всех секторах экономики. Влияние полиэстера на выраженность клеточного и гуморального иммунитет требует комплексного исследования. Важной медико-биологической проблемой являются показатели иммунодефицита, что и было изучено при иммунных токсикологических исследованиях. Одним из научных направлений является изучение влияния полиолов на иммунобиологическую резистентность организма человека. Полученные результаты могут быть использованы для разработки методов повышения неспецифической реактивности.

В настоящей работе рассматриваются механизмы нарушений клеточного и гуморального иммунитета у мышей и крыс после длительного перорального введения Лапрола-604. Показано, что пероральное введение Лапрола-604 приводит к угнетению клеточного и гуморального иммунитета у животных. Исследование было проведено в рамках научно-исследовательской темы Харьковской медицинской академии последипломного образования «Клеточно-молекулярные механизмы воспаления, связанные с хроническими заболеваниями» (номер государственной регистрации 015U001186).

Ключевые слова: полиэстер, Лапрол-604, гуморальный иммунитет, клеточный иммунитет, селезёнка, лимфатические узлы.

Introduction. Nowadays priority tasks of medicine are the health of population and improvement their resistance to adverse social and environmental factors. The organic synthetic factories are the main harmful chemical pollutants, during the last 20-30 years. According to the volume and range, the polyester production is second after detergents [6; 7; 8].

This class of compounds are widespread in the production and life. The polyester influence on the cellular and humoral immunity has been required an integrated research. The actual medical-biological problem is immune deficiency indicators and they need to substantiate during immune toxicological research.

Progressive industrial human activities adverse impact on the ecological systems. It leads to disturbance adaptive processes of an organism, appearance immune deficiency and genetic diseases, relapse of chronic pathological processes. If anthropogenic chemical factors have negatively influenced on immunobiological reactivity of the human organism for a long time, the disruption of protective-adaptive mechanisms and different diseases will be appeared [2; 3].

One of the scientific directions is the investigation of polyols influence on immunobiological resistance of the human organism. The results can be used for developing of methods to increase the non-specific reactivity [4; 6; 7].

The aim of the study is the investigation of both cellular and humoral immunity disorders mechanisms in mice and rats after prolonged Laprol-604 oral administration.

Materials and methods. Laprol - 604 - (L-604) is a viscous clear liquid with regulated physicochemical characteristics MM 600. "Methodical instructions of immunobiological assessment the actions of chemicals" (Russian Ministry of Health, 1998) has been used during experiment. Forty adult (CBAxC57BL)F_1, BALB/C mice and forty adult Wistar rats were randomly divided into four groups. Group 1 (10 mice and 10 rats) received orally a 1/10 LD_{50} of Laprol-604 water solution with the help of a metallic tube daily. Group 2 (10 mice and 10 rats) administered orally a 1/100 LD_{50} of Laprol-604 water solution through a metallic tube daily. Group 3 (10 mice and 10 rats) received orally a 1/1000 LD_{50} of Laprol-604 water solution with the help of a metallic tube daily. Group 4 (controls) consisted of 20 intact animals (10 mice and 10 rats) without Laprol-604 administration. The experiment has been performed for 45 days. All experimental procedures were carried out in accordance to the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (http://www. convention.coe.int/treaty/en/treaties/word/123.doc).

According to the method of G. Gurvich, plasmacytic reaction of the spleen and lymphatic nodes of rats was used as an indicator of structural dynamics of the differentiation of immune cells.

Antibody-forming ability of animal organism has been estimated by enhance of hemolysin-producing cells (N.K. Jerne's method). Cellular and humoral immunity's interaction has been investigated with the help of mice (CBAxC57BL)F_1 in conformity with the methodological guideline "Ambient and occupational environments influence on immunobiological reactivity organism". Following the completion of the subacute research the mice were immunized by 0,2 mL of 10% SRBC suspension.

Then mice lymph nodes and spleens were removed to determine the spleen index, the total number of nucleus-containing cells and the number of nucleus-containing cells per milligram of organ tissue, the number of rosette-forming cells, lymphocyte transformation reaction in response to cellular stimulus such as phytohemagglutinin, liposaccharides, specific allergen, neutrophil damage index, hemolysin-producing function of nucleus-containing cells, antibody-forming and antigen-binding function of immune competent cells on the 6-th day. Homotransplantations activity of lymph node cells was analyzed by the suppress process of allogenic endocolony-formating in mice BALB/C. Functional activity of T- and B-lymphocytes was determined by the index of lymph node cells and spleen cells, which were stimulated by phytohemagglutinin and liposaccharide mitogens. The expression of spleen lymphocyte E_1-, Fc-, C_3- receptors has been investigated with the help of the reactions of E- , EA- , EAC-rosette formation. After immunization with sheep red blood cells, in order to study, the amount of antibody-formation spleen mice cells was measured on the 6-th day. The level of radioactive precursors incorporating in vitro ^3H -uridine, ^3H – thymidine, ^{14}C - protein hydrolysate was used to examine the DNA and protein synthesis in myelocytes of intact mice (control group) and mice immunized by sheep erythrocytes. According to hemagglutination test, the mice blood serum has been separated, lymph nodes and spleens were collected for use in the rosette test. The blood serum has been added each test tube. To activate the complement, the blood serum has been placed in a water bath at 56^0C for 30 min, 1% suspension of SRBC has been added to subsequent serum dilutions prepared in microplates and the blood serum was incubated at 37^0C for 2 hours, followed by 4^0C for 18-20 hours. The agglutination blood serum titer (estimated amount of antibodies in the serum) was defined as the highest serum dilution at which agglutination was still present (microscopic assessment: at least 3 agglutinates per 200× high power field). The blood serum of intact animals served as control.

It is known the ability of T cells to bind SRBC to form spontaneous rosettes depends on the binding of glycoprotein structures present on the surface of lymphocytes with SRBC lipids. All the test procedures were performed while the samples were being centrifuged in a cooling system. Spleen cells have been obtained from the spleens. Then these cells were homogenized, placed on Gradisol L (density 1,077 g/ml) and centrifuged. Lymphocytes have been obtained after that their have twice been washed with Hanks solution, centrifuged again and suspended in the medium achieving the final cell count of 2×10^6/ml of culture. Then 1% SRBC suspension with Hanks solution was added to the spleen cell culture. After incubation at 37^0C, the cultures have been placed at 4^0C for 20 hours. After staining with 0.1% crystal violet, the percentage of spleen cells around which rosettes had formed was counted under the microscope. A rosette was defined as a spleen cells have closely been surrounded by three SRBCs.

Statistical analysis of the data was performed using GraphPadPrism 5. Student's test was used to detect differences between independent groups of normally distributed variables; difference between groups was considered statistically significant at $p<0,05$ [5].

Results and discussion. Prolonged oral administration of Laprol-604 led to a significant increase in percentage of mature plasma cells. The first of all, reticular macrophages dominated in the liver, spleen and lymph nodes due to lead role of fixing the foreign antigens. It has also been known, while the antigen are entering in the organism they interact with macrophages, lymphocytes which transmit information for forming humoral (plasmablast) and cellular immunity. The degree of macrophage-plasmocytic transformation of lymphoid tissue, macrophagal-plasmacytosis transformation of lymphoid tissue influence on the level of antibody production. Quantitative indicators reticular plasma cells, both total and by groups, have been changed by xenobiotics in the organs observed. For example, the amount of basophilic reticulocytes, blasts and plasma cells were increased in bone marrow smears of Group 1 rats. The amount of dormant reticulocytes and mature plasma cells were at the control level. Mature plasma cells predominated among immune cells of the thymus, reticulocytes prevailed in the liver, plasma blasts and immature plasma cells dominated in the spleen, plasma blasts predominated in the lymph nodes. The lowest percentage of quiescent cells-reticulocytes has been observed in the thymus, liver and spleen. A basophilic reticulocyte percentage was identical in the spleen, bone marrow and lymph nodes. At the same time, these cells were slightly

less in the liver and thymus. A passing reticulocyte percentage was observed the lowest in the thymus, although ones the highest - in the bone marrow.

It should be noted that amount reticular macrophages were significantly higher in the spleen and lymph nodes than their content in the liver. The lowest blast percentage was observed in the bone marrow and the highest blast percentage was noticed in the liver. Laprol - 604 was able to change the immunobiological organism reactivity has been evidenced by disrupting of differentiation of immune cells. It has been shown by cytogram immune cells analysis. The number of red and white blood cells were reduced, percentage of E-, EA-, EAC - rosette of immune cells were decreased in the spleen and thymus. Results are shown in table 1 and figure 1.

There were increased the neutrophil damage index and lymphocyte blast transformation index.

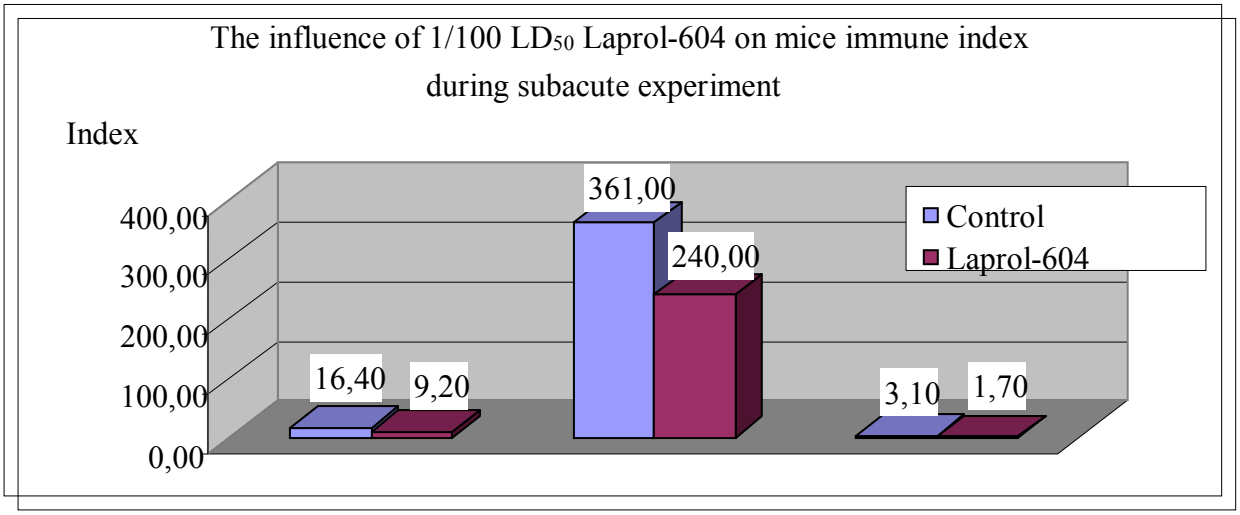

Fig.1 The influence of 1/100 LD_{50} Laprol-604 on mice immune index during subacute experiment

Table 1.

The influence of 1/100 LD_{50} Laprol-604 on mice immune index during subacute experiment

Index	Control	Laprol-604
The count of splenocytes capable of binding sheep red blood cells (formation of rosettes) (to 10^6 cells). Intact animals.	(16,4±1,3) •10^6	(9,2±0,5) • 10^6 *
The count of splenocytes capable of binding sheep red blood cells (formation of rosettes) (to 10^6 cells). Animals immunized by SRBC.	361 ± 15	240 ± 13*
The count of antibody-forming cells in the spleen (to 10^6 cells).	(3,1±0,2) •10^6	(1,7±0,2) •10^6 *

* - $p<0,01$, the significant difference vs control data (one-way ANOVA criteria)

A significant percentage expressing lymphocyte E_1, Fc, C_3- receptors was reduced. The results summarized in table 2 and figure 2.

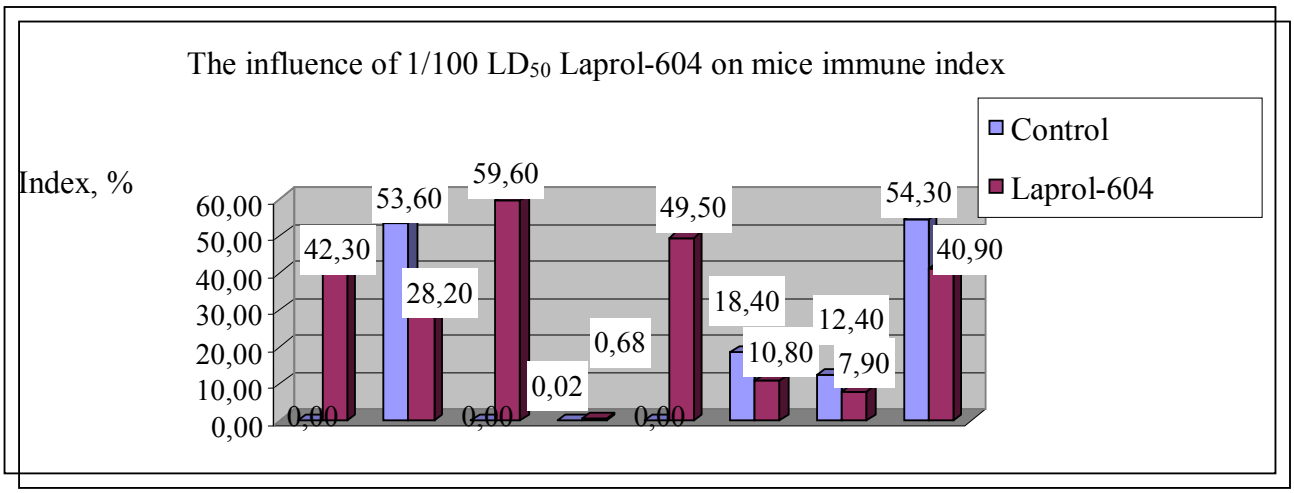

Fig.2 The influence of 1/100 LD_{50} Laprol-604 on mice immune index during subacute experiment

Table 2.

The influence of 1/100 LD_{50} Laprol-604 on mice immune index during subacute experiment

№	Index	Control	Laprol-604
1	Inhibition of antibody formation, %	-	42,3
2	Rosette-forming cells, %	53,6 ± 2,0	28,2 ± 2,5 *
3	Inhibition of phytohemagglutinin induced transformation reaction of lymphocytes, %	-	59,6
4	Neutrophil damage index, %	0,02 ± 0,001	0,68 ± 0,06 *
5	Inhibition of liposaccharide induced transformation reaction of lymphocytes, %	-	49,5
6	Expressing lymphocyte E_1 - receptors, %	18,4 ± 1,3	10,8 ± 0,9 *
7	Expressing lymphocyte Fc-receptors, %	12,4 ± 0,7	7,9 ± 0,5 *
8	Expressing lymphocyte C_3- receptors, %	54,3 ± 2,2	40,9 ± 2,2 *

* - $p<0,05$, the significant difference vs control data (one-way ANOVA criteria)

During the study repoted here, the splenic and thymic index, total number of cell, nucleated cells (NC), the immune cells, and the number of specific lytic concentrations were decreased by Laprol - 604 in 1/10 and 1/100 doses when compared to the control group.

The results are introduced in table 3 and figure 3.

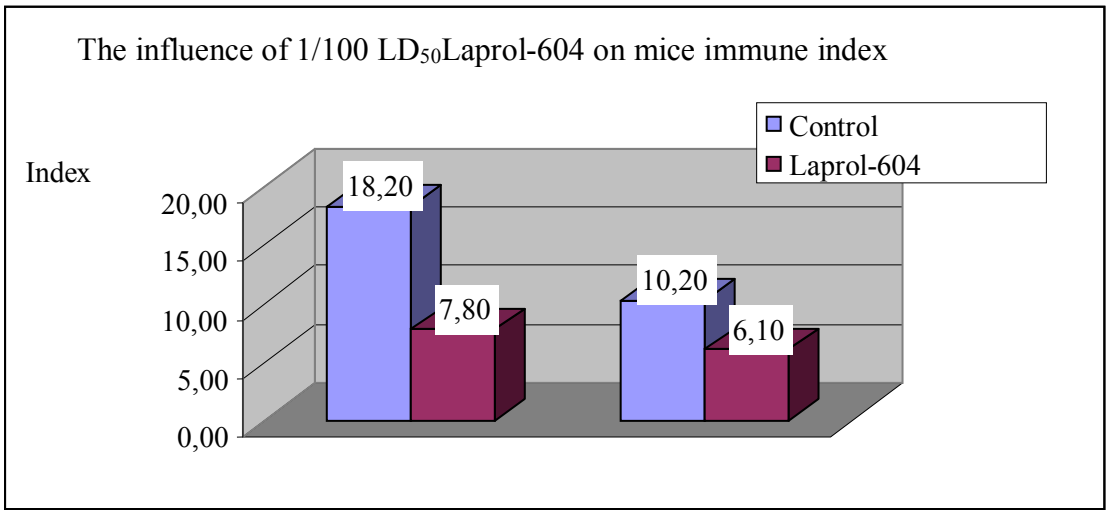

Fig.3 The influence of 1/100 LD_{50} Laprol-604 on mice immune index during subacute experiment

Table 3.

The influence of 1/100 LD_{50} Laprol-604 on mice immune index during subacute experiment

№	Index	Control	Laprol-604
1	Index of phytohemagglutinin induced transformation reaction of lymphocytes	18,2 ± 1,3	7,8± 0,4*
2	Index of liposaccharide induced transformation reaction of lymphocytes	10,2 ± 0,9	6,1± 0,4*

$p<0,05$, the significant difference vs control data (one-way ANOVA criteria)

Immunological observations in animals from group 1 and 2 characterized by inhibit antibody formation, antigen-binding capability of immune competent cells as well as their homotransplantations activity, suppress allogenic endocolony-formation and disturb joint interrelation of cellular and humoral immunity, conditioning the development of immune deficiency.

Protracted Laprol-604 administration reduces the hemolysin-producing function and homotransplantations activity of immune competent cells. Assessment of the immune response to T-dependent antigen SRBC (sheep red blood cells) showed significantly inhibited of T and B - lymphocytes activity and their cooperative interaction in the animals of group 1 and 2. During the study, there was disturbance following processes: the immune cell differentiation, protein and nucleic acid metabolism in the myeloid and lymphoid cells.

Conclusions

1. Within the experimental model reported here, we have shown that protracted intake of Laprol-604 1/10 and 1/100 LD_{50} significantly influences on the animal immunobiological reactivity and disrupts the immune cell differentiation. 1/1000 LD_{50} of Laprol -604 does not provide inhibitory effect on the immune system.

2. 1/10 and 1/100 LD_{50} of Laprol -604 suppress antibody production, antigen-binding ability, disturb the immune cell differentiation and protein and nucleic acid metabolism of myeloid and lymphoid cells. In addition, T and B - lymphocytes activity and their cooperative interaction have been decreased by Laprol -604 for 45 days.

3. The results reported here and the fact that polyols is frequently used in many human life areas to requires the study their influence on the immune system of living organisms.

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Abbreviations

EA - Erythrocyte-amboceptor complex; EAC - Erythrocyte-amboceptor-complement complex; E_1-receptor-bearing cells; Fc-receptor-bearing cells; C_3- receptor-bearing cells; RFC - rosette-forming cells; (S)RBC - (sheep) red blood cells